# Effect of Prostaglandins on Corneal Stiffness and Intraocular Pressure Measurement

Undergraduate Research Final Thesis

Presented in Partial Fulfillment of the Requirements for Graduation with Honor Research Distinction

By

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2013

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## Abstract

Glaucoma is a critical eye condition that would eventually cause vision loss and irreversible blindness due to the damage of optic nerves. High intraocular pressure (IOP) is frequently associated with this disease and hence often used as a diagnostic parameter for screening and management of glaucoma. Topical anti-glaucoma drug, Prostaglandin, has been shown to be effective in lowering IOP with few side effects. However, it was also found that Prostaglandin could alter the structural properties of ocular tissue such as the cornea. Goldmann Applanation Tonometry (GAT), which has been the clinical standard in measuring intraocular pressure in glaucoma patients, is used with assumptions that the structural and biomechanical properties of cornea are negligible. The change in corneal properties due to drug effect may thus directly interfere the IOP measurements, resulting in artificially low IOP readings. Therefore, the purpose of this study is to investigate the potential influence of prostaglandin drugs on cornea stiffness and the IOP measurement. This is accomplished by treating canine eyes with Prostaglandin analog, Bimatoprost at two different concentration; 200nM and 500nM, and vehicle solution was applied to the contralateral eyes as controls. GAT was used to measure the IOP of both the treated and control eyes. Mechanical testing was then performed on dissected corneal strips to determine the treatment effect of the drug on the corneal stiffness. The experimental results (n=12) showed that there was a significant reduction in corneal

stiffness in lower concentration group but not in the higher concentration group. There was no significant difference found in the GAT readings relative to reference IOPs and the change of thickness before and after testing was minimal. This suggested that the IOP measurement was not significantly altered after a one-day administration of Bimatoprost. However, the drug effect was significant only in the lower concentration group proposed that there was some confounding factors in the higher concentration group. This result could also suggest that the drug effect was more effective at low concentration. One of the limitations of this study was the small sample size. Therefore, a larger sample size and also a longer drug exposure time would be required to verify the drug effect on corneal stiffness and its influence on IOP measurement. Although current study did not demonstrate a strong evidence of the drug effect on corneal stiffness and IOP measurement, it provided some preliminary data for research direction as well as a good framework for future work.

## Dedication

Dedicated to my family, Walter, Joyce, Yi Wen, Yi Ling, Zheng Yi and Jiunn Xhiong.

A special dedication to the dogs that has contributed to this project.

## Acknowledgments

I would like to thank the College of Undergraduate for the Undergraduate Research Scholarship, the Franklin dog shelter and adoption center to provide the eye samples.

I would like to express my deep gratitude to Professor Jun Liu for all the support, guidance and assistance throughout my entire research project. Her support has been my main motivation to complete my research project. I would also like to thank Dr. Hugh Morris for the professional help and constructive advice and technical assistance, Professor Cynthia Roberts for being my oral exam committee and some great advices and suggestions for the project.

Special thanks give to Benjamin Cruz Perez for the technical assistance and moral support as well as Dr. Junhua Tang for technical assistance.

Finally, I wish to thank my parents, Mr. and Mrs. Liew for their love, support and encouragement in my study

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# Chapter 1: Introduction

Glaucoma is a critical eye condition that would eventually cause vision loss and irreversible blindness due to the damage of optic nerve. High intraocular pressure (IOP) is often, but not always associated with this disease. Therefore, IOP measurement is frequently used as a diagnostic parameter for screening and management of the disease. Goldmann Applanation Tonometry (GAT) has been the clinical standard in measuring IOP with the underlying principle that assumes that the structural and biomechanical properties of cornea are negligible.

Nonetheless it was shown that topical use of anti-glaucoma drugs, prostaglandin (PG) reduces central cornea thickness (CCT) and induces morphological as well as biochemical changes in cornea stromal cells.[1, 2] Since structural and biomechanical properties of the cornea will be greatly affected by the medication[3, 4], this suggests that PG treatment might indirectly cause IOP measurement errors. The clinical implications of this are significant because the reduction of IOP in PG-treated patients might not reflect the actual improvement in the disease but the softening of cornea. Recent experimental studies have shown a strong correlation between GAT errors and corneal tensile modulus, which is consistent with the theoretical prediction that suggested that the differences in corneal biomechanics in fact, have greater impact on IOP measurement errors than corneal thickness and curvature.[5, 6]

This study is therefore intended to investigate the potential influence of prostaglandin drugs on cornea biomechanical properties and the IOP measurement.

Objective:

(1) To investigate the effect of Prostaglandin on the cornea stiffness

(2) To examine the effect of Prostaglandin on IOP measurement

(3) To examine the reduction in collagen content after prostaglandin treatment

# Chapter 2: Methodology

## Sample Preparation

First, pairs of fresh canine eyes (N=12) were obtained from Franklin Dog Shelter and Adoption Centre and enucleation was completed within one hour postmortem. Before dissecting, CCT was measured from the whole globe using ultrasound pachymeter. After sterilizing the globes with Provodin-iodine topical antiseptic, the canine eyes will be cut at the equator to separate the anterior and the posterior segments. The eyes were dissected carefully by cutting around the limbus of the eyes leaving sufficient sclera to be mounted on the anterior chamber and mechanical testing. For mechanical testing purposes, more sclera will be spared at nasal-temporal direction. The corneal buttons were then rinsed properly and placed in wells with culture mediums.

***Ingredients for cornea preserve medium:***

DMEM: 100mL

Chondroitin sulfate: 1.35g

Dextran: 8g or P188: 5mL

Nonessential amino acids: 1 mL

Fetal bovine serum (FBS): 2 mL

Penicillin-streptomycin: 1 mL

HEPES buffer: some to adjust the pH

Prostaglandin (PG) analogue, Bimatoprost Treatment

The culture medium was replaced with fresh medium supplemented with 200nM and 500nM of Prostaglandin analog, Bimatoprost. In this study, Bimatoprost (Lumigan 0.01%) was used because this is one of the most potent IOP-reducing medications.[8] For each pair of canine eyes, either eye was placed into vehicle solution and contralateral eye was immersed into medium supplemented with Prostaglandin to observe the treatment effect. PG concentrations were chosen based on the protocols used in Weinreb 's studies on effect of Latonoprost on scleral permeability.[16.] The cornea in culture medium was then incubated at 37°C for 24 hours. The calculations below illustrated the volume of Bimatoprost to be added into the culture medium to achieve the desired concentrations.

***Calculations:***

Concentration of Bimatoprost = 0.1 mg/mL = 0.1 g/L

Given: Molecular Weight = 415.58 g

$$\text{Concentration of Bimatoprost } = \frac{0.1\frac{\text{g}}{\text{L}}}{415.58\frac{g}{mol}} = 2.4063 \times \frac{10^{-4} mol}{L}$$

$$= 0.24063\ mM$$

**Target Concentration: 200nM and 500 nM**

$M_1V_1 = M_2V_2$ ($V_1$ = volume of drug; $V_2$= volume of preserve medium)

$0.24063 \times 10^{-3}\ V_1 = M_2\ (V_2+V_1)$

Using cornea preserve medium, $V_2$ = 50 mL

| **For 200 nM:** | **For 500 nM:** |
|---|---|
| $M_2 = 200 \times 10^{-9}$ M | $M_2 = 500 \times 10^{-9}$ M |
| $V_1 = 41.592$ µL | $V_1 = 104.110$ µL |

Goldmann Applanation Tonometry

CCT was measured before testing. The cornea was taken out from the wells and mounted on anterior chamber for GAT measurement. The experimental setup will be similar to the bovine anterior segment perfusion culture model. [7] The anterior chamber used was shown below (Figure 1). One of the channels of the anterior chamber was connected to the column filled with culture medium to prevent swelling and another to pressure sensor that confirmed and monitored the real-time pressure level in the chamber.

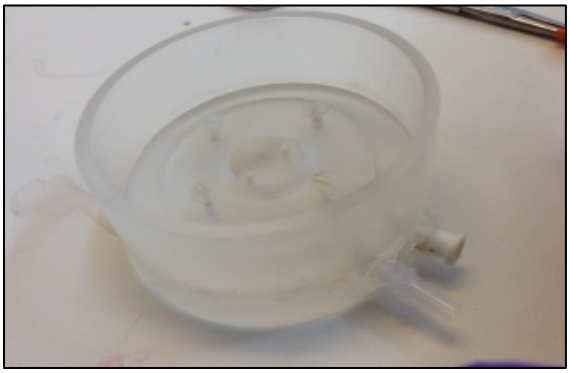

Figure 1: Anterior chamber for cornea mounting

The IOP measurement protocols was modified from Goldmann applanation techniques reported by Tang et al.[11] Before applanation, the anterior segment was secured in a holder to keep it stationary. Fluorescein dye was applied gently to the cornea surface using a sterilized cotton tip to help visualized the correct alignment of Goldmann mires under slit lamp examination. Pressure sensor was placed at the same level as the anterior chamber. Three pressure levels; 20mmHg, 30mmHg and 40 mmHg were tested. The

pressure was altered by adjusting the height of the column. To get GAT readings, the tonometer knob was adjusted to make the inner edge of two semicircles slightly touching. Each measurement was repeated three times to get an average. The GAT readings were then recorded.

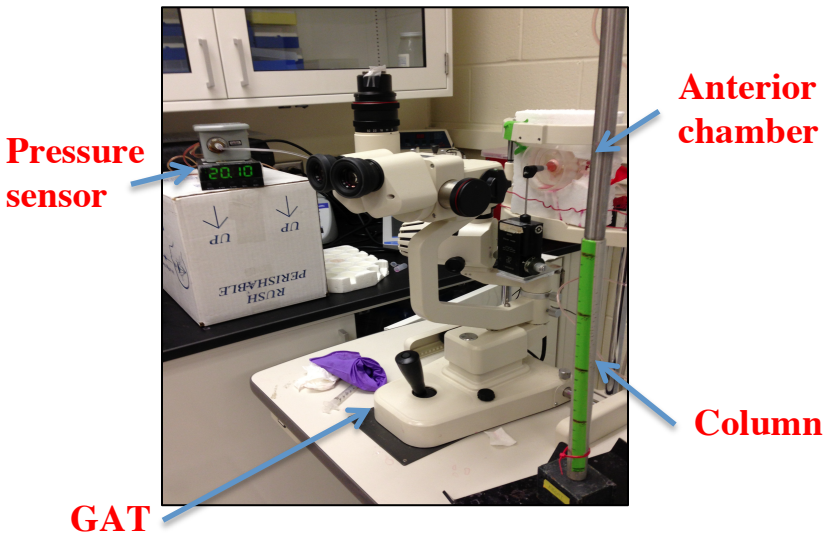


Figure 2: IOP measurement using GAT setup in ex- vivo eyes

Mechanical Testing: DMA and Ramp Analysis

To measure corneal stiffness, a uniaxial tensile test was performed after IOP measurement. The protocols were similar to method used by Tang et al.[11] The corneal strips were excised in the nasal-temporal direction. Sample widths and thicknesses of tissues were measured using ultrasound pachymeter before testing. The corneal strip was then placed between two testing grips. Half of the sclera was left above the upper grip and the part in between the grips was only cornea. The gap distance was adjusted to apply

force on the tissue. The cornea was pre-conditioned before mechanical testing. Two types of tests were performed; DMA and Ramp. DMA analysis involved a cyclic loading while ramp analysis included uniaxial tensile load. The Rheometric System Analyzer (RSA) computed the stress and strain. The experimental data were then saved and analyzed later using Matlab simulations.

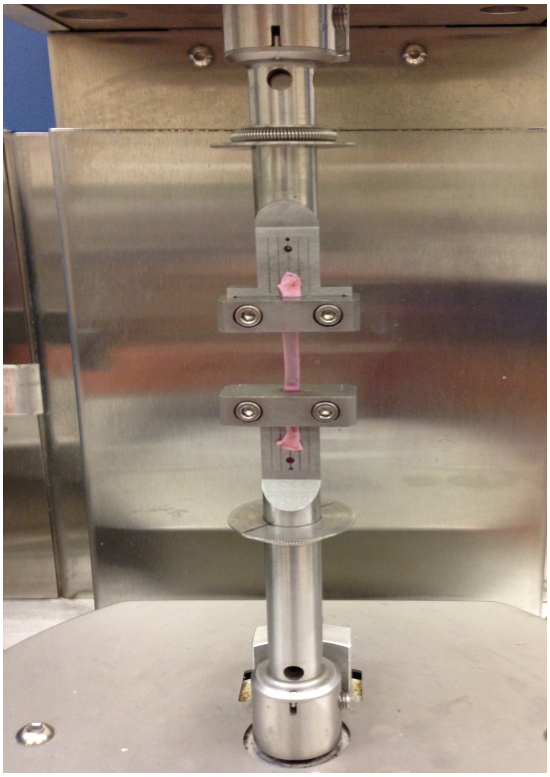

Figure 3: Mechanical testing setup

# Chapter 3: Results and Discussion

## Goldmann Applanation Tonometry (GAT)

The GAT readings of three different pressure levels, 20 mmHg, 30mmHg and 40 mmHg were compared with reference IOPs to detect the difference. In the lower drug concentration group (200nM), the mean GAT readings were slightly lower for every pressure levels in the treated eyes as compared to control eyes. After performing a student paired t-test on the control and treatment group, it was found that there were no significant difference since the p-value for all three pressure levels exceeded p=0.05 (Table 1 & Figure 1). In the higher concentration group, the reverse trend was observed (Figure 2). The mean GAT readings in the treated eyes were surprisingly higher than the control groups. However, the differences were also not significant (p>0.05) (Table 2).

Table 1 GAT readings relative to reference IOP in 200nM group

| N=6 | **GAT (20mmHg)** | **GAT (30mmHg)** | **GAT (40mmHg)** |
|---|---|---|---|
| **Treated** | 11.88±2.68 | 21.33±4.26 | 31.06±3.66 |
| **Control** | 13.72±1.78 | 22.78±3.46 | 31.27±4.54 |
| P-value | 0.122 | 0.287 | 0.474 |

Table 2 GAT readings relative to reference IOPs in 500nM group

| N=6 | **GAT (20mmHg)** | **GAT (30mmHg)** | **GAT (40mmHg)** |
|---|---|---|---|
| **Treated** | 12.89±5.01 | 21.5±5.86 | 29.50±7.85 |
| **Control** | 11.33±4.81 | 20.2±6.77 | 28.44±9.17 |
| P-value | 0.243 | 0.269 | 0.384 |

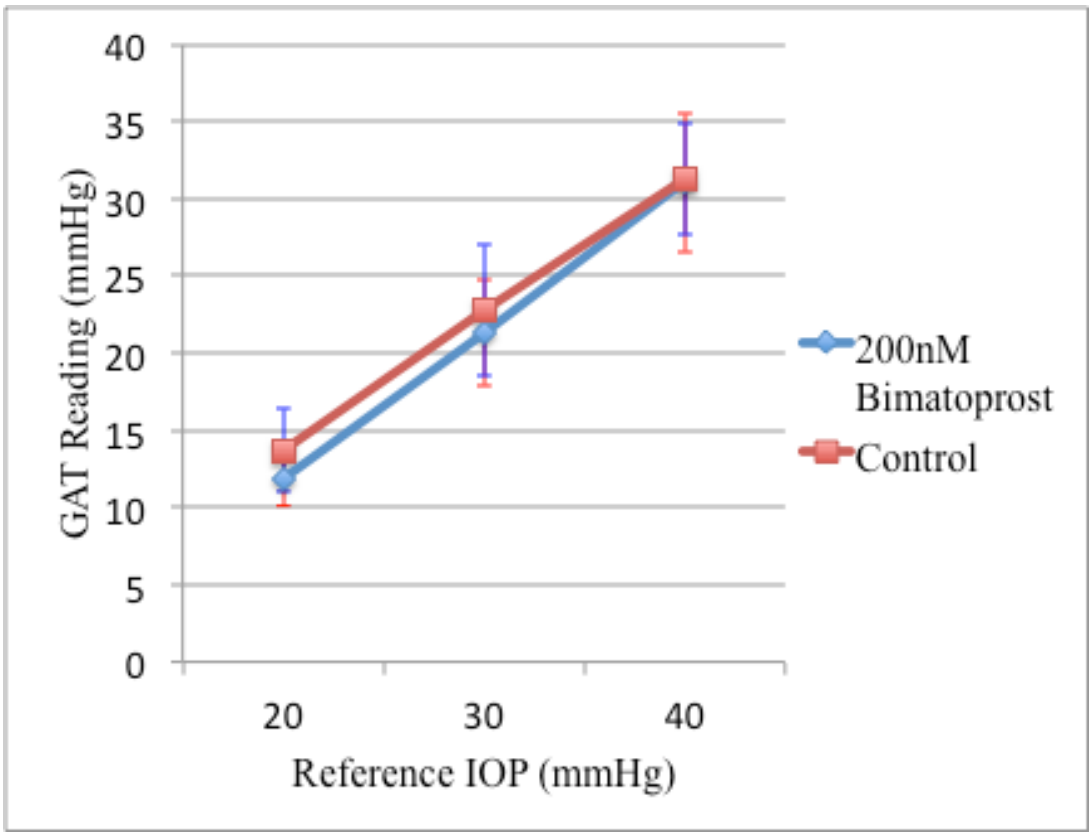


Figure 4 GAT readings vs. Reference IOPs in lower drug concentration

In general, the GAT readings for canine eyes were generally 10mmHg lower than the reference IOPs (Table 1 & Table 2). By observing the trend from the graph (Figure 1), the mean difference was greater in lower pressure levels and the mean GAT values in higher-pressure levels converge. Comparing the standard deviation of the mean GAT values (Table 1), it was observed that the range also increased with the pressure.

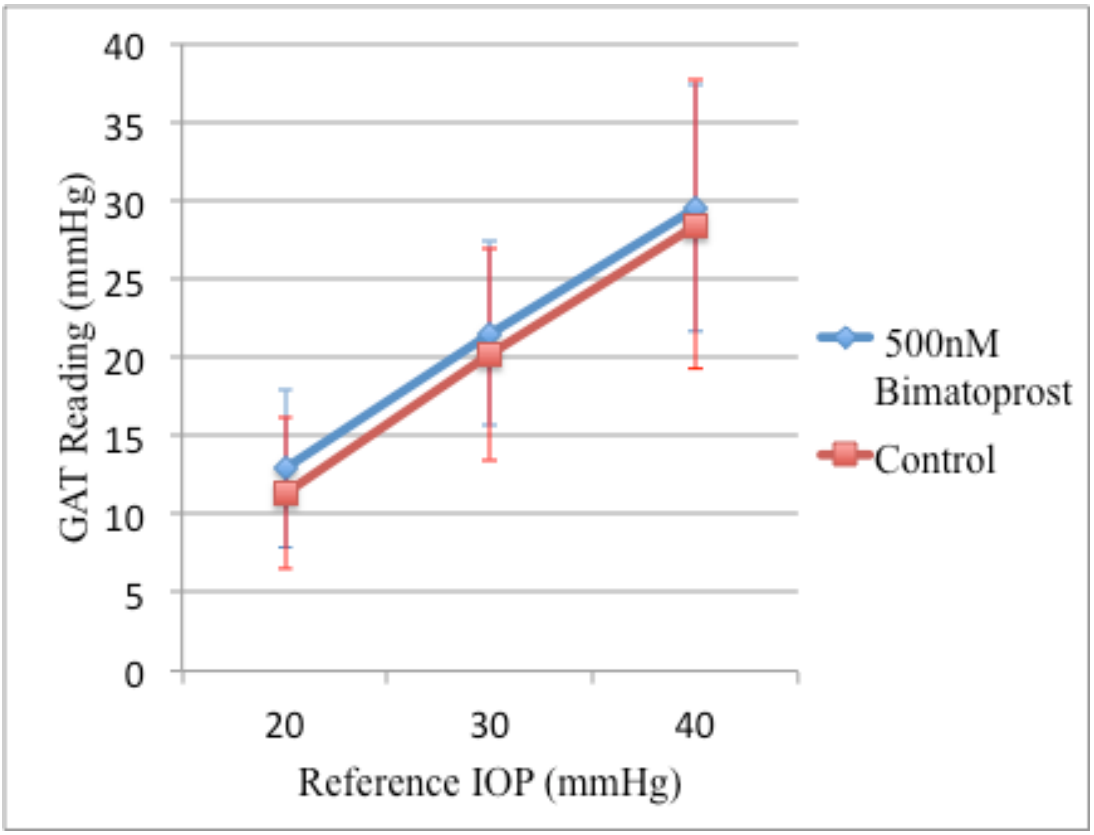


Figure 5 GAT readings vs. Reference IOPs in higher drug concentration

In higher drug concentration (500nM), the mean GAT readings consistently showed slightly higher values in treated eyes as compared to control. Similar to the lowe concentration group, the standard deviations increased with higher-pressure levels (Table 2).

Mechanical Testing: Corneal Stiffness

After performing dynamic mechanical analysis and ramp analysis, it was observed that 200nM group showed a reduction in complex modulus and secant modulus in prostaglandin-treated eyes (Table 3). The damping ability, tan **(**δ) increased after treatment. These changes were significant ($p<0.05$) between the treated and control group, suggesting a treatment effect of Bimatoprost.

Table 3 Complex modulus, tan delta and secant modulus in lower concentration group

| N=6 | **Complex Modulus (MPa)** | **Tan (δ)** | **Secant Modulus (MPa)** |
|---|---|---|---|
| **Treated** | 5.33±1.42 | 0.18±0.042 | 6.95±2.95 |
| **Control** | 6.24±1.90 | 0.14±0.011 | 9.53±2.92 |
| P-value | 0.023* | 0.042* | 0.033* |

Note: * means $p < 0.05$

Exposure to higher concentration (500nM) of Prostaglandin showed no significant difference ($p>0.05$) in the complex modulus, tan **(**δ) and secant modulus (Table 4). However, by looking at the trend on the graph (Figure 3), the average of complex modulus and secant modulus were higher in treated than the control samples. The average damping ability, tan **(**δ) of the tissue exposed to 500nM Bimatoprost was lower in treated group.

Table 4 Complex modulus, tan delta and secant modulus in higher concentration group

| N=6 | **Complex Modulus (MPa)** | **Tan (δ)** | **Secant Modulus (MPa)** |
|---|---|---|---|
| **Treated** | 5.63±1.99 | 0.15±0.018 | 7.74±3.57 |
| **Control** | 4.63±0.92 | 0.17±0.015 | 6.56±1.99 |
| P-value | 0.078 | 0.110 | 0.170 |

Looking at the figure shown below, two concentration groups actually showed opposite trend, which is consistent with results from GAT. However, the higher concentration group did not show significance while lower concentration group displayed significant reduction in stiffness and increased damping ability.

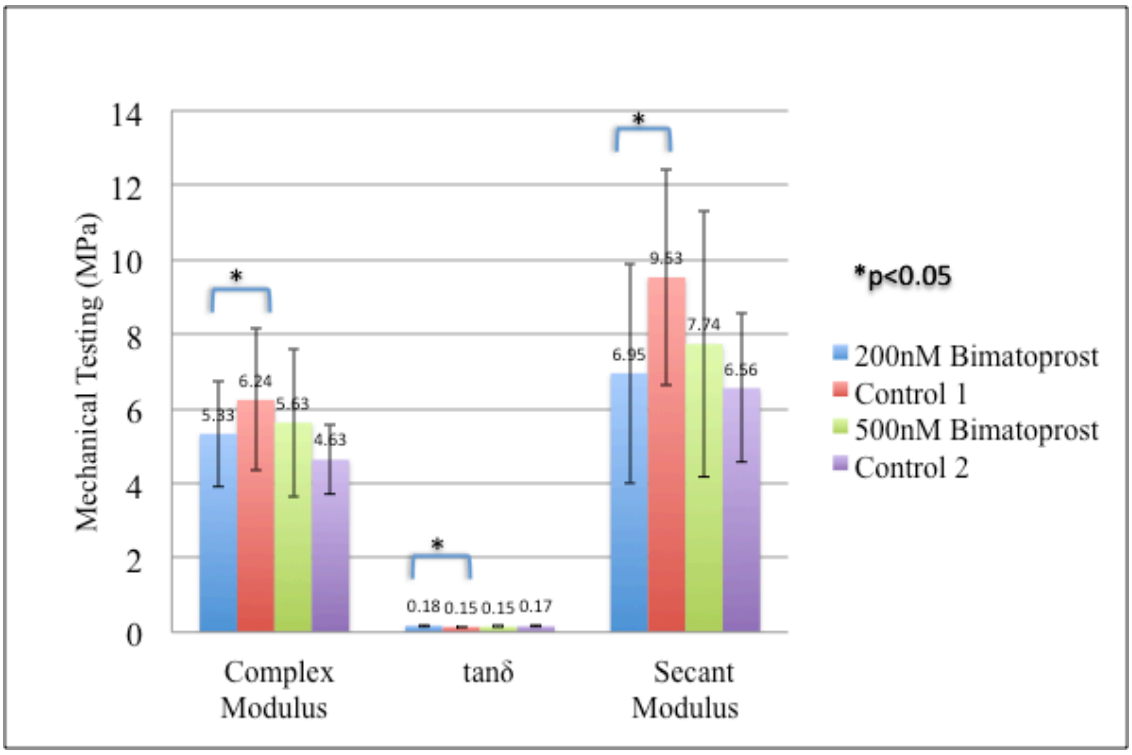


Figure 6 Comparison of properties between two concentration groups

Change in thickness

The thickness measurement prior to and after testing showed no significant difference ($p>0.05$) in both control and treated group for lower drug concentration. Nevertheless, the average thickness reduced slightly before and after testing in general (Table 5). In treated group, the standard deviation was consistent before and after testing. In comparison, it was found that the standard deviation for the thickness was really large after testing.

Table 5 Group 200nM: Thickness measurement before and after testing

| **Thickness (N=6)** | Treated (μm) | Control (μm) |
|---|---|---|
| Before | 654.22±103.01 | 692.95±69.41 |
| After | 643.11±112.73 | 652.38±138.89 |
| P-value | 0.394 | 0.199 |

In higher concentration group, the same trend was observed. There were no significant difference ($p>0.05$) but the thicknesses in both treated and control group were reduced as well after testing (Table 6).

Table 6 Group 500nM: Thickness measurement before and after testing

| **Thickness (N=6)** | Treated | Control |
|---|---|---|
| Before | 680.2±68.72 | 657.17±73.52 |
| After | 642.78±60.39 | 650.00±64.40 |
| P-value | 0.107 | 0.372 |

On the other hand, when comparing the treated and control groups in both concentrations, it was observed that after testing, prostaglandin treatment at two concentrations caused not much difference in the thickness. The thickness before testing was not compared here because the measurements were done before prostaglandin treatment and the thickness difference simply reflected the variation of corneal thickness in nature.

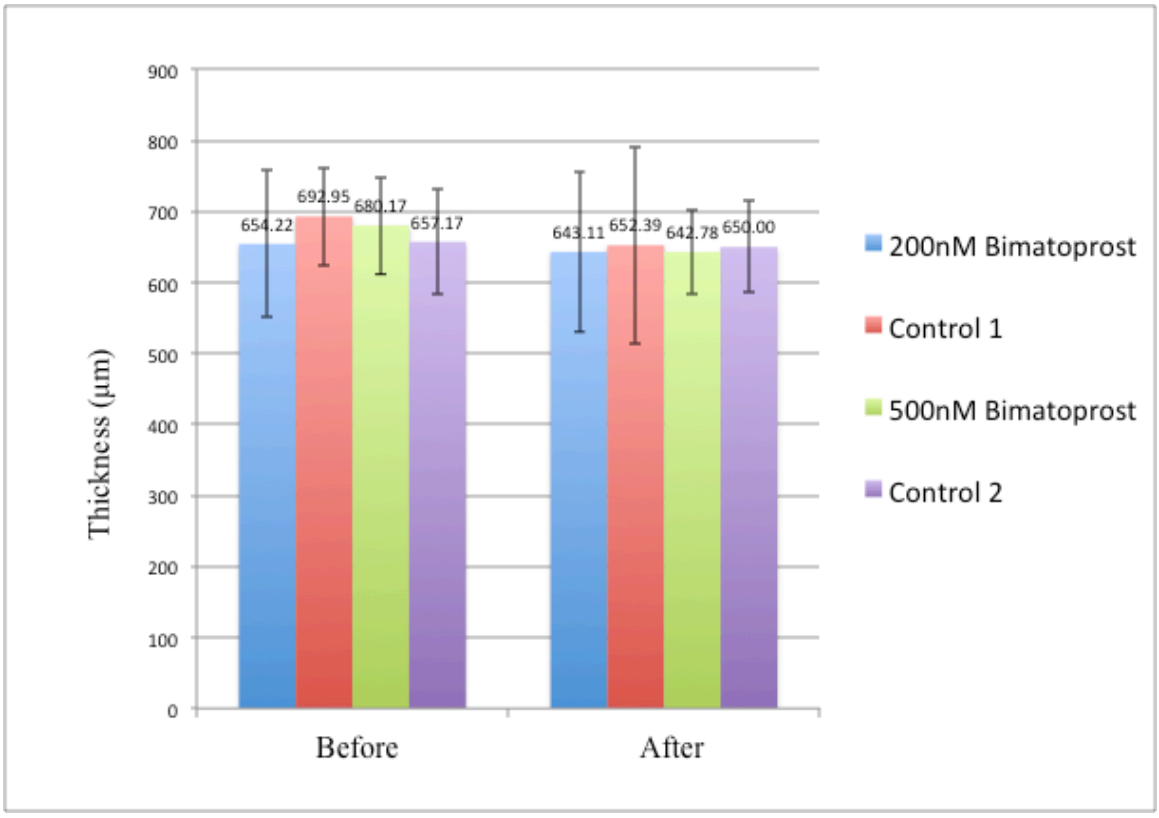


Figure 7 Change in thickness measurement before and after testing

## Discussion

The GAT readings consistently showed that the GAT readings in canine eyes were about 5-10 mmHg lower than the reference IOP. This observation was supported with Tang et al. studies that have also reported the large underestimation of IOP in canine eyes due to distinct biomechanical properties from human eyes.[6] Since there was no significant difference in GAT readings across treated and control group, this suggested that the treatment effect of Prostaglandin analogue, Bimatoprost on IOP measurement was not observable after drug administration for 24 hours. In addition, the opposite trend observed between two different drug concentrations indicated high variability in the measurement. This might be partly due to operator's subjectivity in reading the GAT measurements. In addition, the swelling of canine eyes caused blurred edges of semicircle, making the measurements essentially difficult. It was challenging to measure IOP on ex-vivo eyes because the accuracy of measurement was also affected by the techniques that have been used to mount the cornea on the anterior chamber. Since there was a fixed window size on the chamber, corneas with different sizes could have different boundary conditions. Since the cornea is thinnest in the center and its thickness increases radially, the size of the window on the anterior chamber caused the applanated area essentially different for corneas with distinctive sizes. This could indirectly contribute to more variation in the GAT readings. On the other hand, the upright placement of the chamber also created difficulty in keeping the entire cornea moist, as solution would accumulate at the bottom part of the corneas.

From the experimental results of lower concentration group, the effect of Bimatoprost on corneal modulus was significant. The reduction in corneal stiffness was consistent with the results found in a study that showed significant increase in matrix metalloproteinase (MMP) activity and decrease in collagen type 1 in corneal stromal later after prostaglandin treatment on rabbit corneas for two months.[9] It was known that MMP is highly responsible for regulation and maintenance of the extracellular matrix (ECM) and high MMP activity in cells cause degradation of collagen fibrils. The reduction in collagen content might correlate with the lower stiffness observed in the prostaglandin-treated eyes.

However, there could be an alternate explanation for observed treatment effect in the low drug concentration group. It was shown that Bimatoprost also reduced central corneal thickness; the decrease in thickness might cancel off the effect of corneal swelling. Since the control group did not experience the de-swelling effect from the drug, a higher stiffness was found for swollen tissues. The smaller modulus found on treated group actually reflected unchanged stiffness in normal condition of the cornea due to the cancelling effects while stiffness of the control group was elevated due to swollen corneas.

The experimental result showed no significance found on higher concentration group (500nM). There was no known reason for the increased stiffness on treated eyes. One of the possible reasoning is that Bimatoprost is more effective at low concentration. Literature showed that only small concentration of Bimatoprost was found in aqueous humor of the treated eyes. The maximum concentration of Bimatoprost was found to be

6.81 ± 1.36 nM one hour post dose and the drug free acid form was found to be maximum after 2 hours post dose and had a concentration of 30.9 ±16.41 nM.[17] This indicated that drug effect could actually work better in a lower concentration profile as compared to high concentration. However, the drug mechanism in corneal cells remained largely unknown at this point. Although there were contrasting features found between the two different concentrations, it was less likely for Bimatoprost to stiffen the corneas. Therefore, this suggested the presence of artifacts in high concentration group and a confounding factor has yet to be identified. In order to confirm and investigate the underlying reason, more experimental data would be required for further detailed analysis.

# Chapter 4: Conclusions and Future Work

From my studies, it was found that there was no significant difference found in the intraocular pressure measurement using Goldmann Applanation Tonometry after Prostaglandin treatment. Although the GAT readings showed opposite trend for two different concentrations, it was greatly due to the high variability in the measurements. Both concentrations revealed a large standard deviation in IOP measurement and the difference between the treated and control eyes fell well in that range. Therefore, it could not be concluded that if the treatment effect caused a deviation from the reference pressure readings. In addition, there were some limitations in the GAT measurement as the swollen cornea posed a challenge in matching the blurred inner edges of the semicircles. Moreover, GAT measurement relied highly on the operator as a human error of a few mmHg might be induced in adjusting the tonometer knob. In the future, a different technique such as a portable Tonopen could be used to measure the IOP in addition to the GAT. Since Tonopen have a smaller surface area of contact with the cornea as compared to the GAT, the issues of boundary conditions could be eliminated and induced fewer errors in the measurement. Also, the drug effect might not be apparent after a 24 hours-treatment as the cellular mechanism in response to the drug was shown to happen in 24 to 72 hours period. Therefore, a longer treatment time could be done in the future in order to observe a more obvious drug effect.

On the other hand, a statistically significant correspondence was observed between the treated eyes and a reduction of stiffness in the lower drug concentration group. The complex and secant modulus were both lower in the treated than the control

group suggesting that drug effect might actually contribute to cornea softening. However, with an opposite trend found on the higher concentration, the drug effect could not be confirmed yet. A disparity in experimental results for two different concentrations suggested that more drug concentrations should be tested for a better understanding of the drug mechanism. A larger sample size should be used in the future to eliminate the variability between eyes and it would be easier to identify the confounding factors with more experimental data.

Moreover, a histology procedure or collagen assay technique could be used for quantification of corneal collagen content in order to confirm the correlations found with reduction in corneal stiffness. Since the corneal stromal cells were largely composed of collagen, the collagen fibrils in the corneas are highly responsible to withstand the external loads imposed on them.[15] If a reduction in collagen content were observed in the corneal cells, the decrease in stiffness would be confirm and better supported. This would help to eliminate the alternate explanation that suggested that the smaller modulus was due to the de-swelling effect of the treated group since the reduction in corneal stiffness hypothesis was merely based on a relative measurement.

The experimental result in this study also showed no significant difference in the thickness before and after testing. Although this suggested that swollen cornea might not be a confounding factor in the study, the swelling could be masked by the prostaglandin effect on central corneal thickness and the presence of variability within the same pair of eyes. With the small sample size (n=12) used in this study, it was difficult to assure that the swelling effect was eliminated.

For future experiment, cell viability of the cornea should also be assessed to evaluate the drug effect on the sustainability of living cells in the culture medium since the cellular response to the drug is critical to cause the change in corneal biomechanical properties.

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